Euphytica **136:** 45–50, 2004.

Heritability of resistance to *Aphanomyces euteiches* races 1 and 2 in alfalfa

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Received 25 February 2003; accepted 5 December 2003

Key words: alfalfa, *Aphanomyces euteiches*, heritability of resistance, root rot

Summary

Aphanomyces euteiches is a soilborne plant pathogen that causes root rot in several leguminous crops including pea, bean and alfalfa. In alfalfa, at least two distinct races of the pathogen have been identified. Although many alfalfa cultivars are resistant to the race 1 isolate of *A. euteiches*, resistance to race 2 isolates of the pathogen is lacking in the great majority of cultivars. Our objectives were to calculate heritability estimates of resistance to *A. euteiches* races 1 and 2 in alfalfa. These estimates will be useful in predicting gain from selection for disease resistance. Three different alfalfa populations developed from the cultivars 3452 ML, Affinity + Z and Depend + EV were examined in this study. Each population consisted of 32 randomly selected half-sib families. Heritability on a half-sib progeny means basis was calculated based on data from pathogenicity tests conducted under greenhouse conditions. Confidence intervals were calculated for each heritability estimate. Heritability estimates based on experiments conducted over two years were high for all populations, ranging from 0.84–0.90 for resistance to *A. euteiches* race 1 and from 0.62–0.66 for resistance to *A. euteiches* race 2. These results suggest that improving levels of resistance to both races of the pathogen should be possible through selection.

Introduction

The oomycete *Aphanomyces euteiches* Drechs. causes severe root rot of several legumes including pea (*Pisum sativum* L.) (Papavizas & Ayers, 1974), common bean (*Phaseolus vulgaris* L.) (Pfender & Hagedorn, 1982) and alfalfa (*Medicago sativa* L.) (Delwiche et al., 1987). At least seven pathotypes of *A. euteiches* exhibiting differential pathogenicity to specific legume species have been identified (Pfender & Hagedorn, 1982; Grau et al., 1991; Holub et al., 1991). Within the alfalfa pathotype of *A. euteiches*, strains have been classified as either one of two races based on the disease response of resistant and susceptible check populations (Fitzpatrick et al., 1998). Disease in alfalfa is characterized by stunted seedlings with chlorotic cotyledons, damping off and poor stand establishment (Grau, 1990). The pathogen may also cause a sub-lethal disease that results in chronic root infection of mature plants (Grau, 1990). Other than host plant resistance, no effective control for this disease of alfalfa is available. To minimize losses, the cultivation of resistant alfalfa cultivars is recommended, along with the avoidance of poorly drained and heavily infested fields (Grau, 1990). Several studies indicate that alfalfa cultivars with resistance to *A. euteiches* exhibit significantly better seedling health, yield, and persistence than varieties with low resistance when grown in naturally infested soils (Wiersma et al., 1995, 1997; Munkvold et al., 2001).

Alfalfa populations are typically evaluated for reaction to *A. euteiches* using a standardized test protocol established by the North American Alfalfa Improvement Conference Committee on Standardized Tests. The standard test uses a scale of 1 to 5 for evaluating disease severity, where 1 = healthy plant and 5 = dead plant (Fitzpatrick et al., 1998). Plants scored as either a 1 or 2 (slight necrosis of roots and hypocotyls) are considered resistant (Fitzpatrick et al., 1998). Resistance is rated according to a scale based on data from standard tests (Alfalfa Council, 2002). Populations are considered to have high resistance (HR) if more than 50% of the plants are resistant. Populations with 31–50% resistant plants are rated as resistant (R). Populations with 15–30% resistant plants are rated moderately resistant (MR) and those with 6–14% resistant plants are considered to have low resistance (LR). Only populations that have less than 6% resistant plants are rated susceptible (S). At present, 71.4% (135/189) of the available certified alfalfa cultivars with a fall dormancy rating of 2 to 4 are rated as R or HR to race 1 of *A. euteiches* (Alfalfa Council, 2002). However, only 3.2% (6/189) of these varieties are rated as R or HR to race 2 of *A. euteiches* (Alfalfa Council, 2002).

The genetic basis of resistance to *A. euteiches* is best characterized for pea. Shehata et al. (1983) scored resistance to *A. euteiches* in a greenhouse trial using a disease index scale of 1 (healthy roots) to 6 (severe root rot and dead plant). Frequency distributions for disease index ratings among three different F_2 populations suggested that resistance was inherited quantitatively. Broad sense heritability estimates ranged from 0.45–0.57 (Shehata et al., 1983). Pilet-Nayel et al. (2002) evaluated resistance in the field to *A. euteiches* in a pea population grown over two years in two different environments. Heritability of resistance on a progeny mean basis was estimated at 0.30 using a root rot index of 1(healthy) to 5 (severe root rot, dead plant).

The inheritance of resistance to *A. euteiches* in alfalfa has not been characterized. However, evidence suggests that resistance to race 1 and race 2 of *A. euteiches* is controlled by different genes. The population WAPH-1 is the resistant check in standard tests for evaluating resistance to race 1 of *A. euteiches*, with approximately 50% resistant plants expected (Fitzpatrick et al., 1998). However, in standard tests for evaluating resistance to race 2 of *A. euteiches*, WAPH-1 is a susceptible check, along with the cultivar Saranac, with approximately 2% resistant plants expected (Fitzpatrick et al., 1998). Additionally, among the 135 certified alfalfa cultivars with a fall dormancy rating of 2 to 4 that are rated as R or HR to race 1 of *A. euteiches*, only 6 are rated as R or HR to *A. euteiches* race 2 (Alfalfa Council, 2002). The objectives of this study were to estimate heritability of resistance in three different alfalfa populations to both race 1 and race 2 of *A. euteiches*. Heritability estimates will be useful in predicting gain from selection for disease resistance.

Materials and methods

Pathogen isolates and plant materials

Two different isolates of *A. euteiches* were used in this study, *A. euteiches* MF-1, considered the type isolate of race 1, and *A. euteiches* NC-1, the type isolate of race 2 (Fitzpatrick et al., 1998). The cultures were maintained on potato dextrose agar (PDA, Difco Inc., Detroit, MI) at room temperature.

Three different alfalfa populations were used in this study. Population 1 was the cultivar Affinity + Z, population 2 was the cultivar Depend + EV and population 3 was the cultivar 3452 ML. All three cultivars have been rated as being resistant (R) to *A. euteiches* race 1 (Alfalfa Council, 2002). The resistance classification of the three cultivars with respect to *A. euteiches* race 2 has not been determined.

Inoculations and evaluation of disease severity

For each population, 32 half-sib families were evaluated in two independent experiments, one for resistance to *A. euteiches* race 1 isolate MF-1 and the other for resistance to *A. euteiches* race 2 isolate NC-1. Each experiment was conducted twice, once in 2002 and once in 2003. Each experiment involved a six-replicate randomized complete block design with eight seedlings per half-sib family in each replicate. The standard test protocol for evaluating resistance in alfalfa to *A. euteiches* (Fitzpatrick et al., 1998) was followed in this study. Briefly, seeds were first mixed in a commercial preparation of *Rhizobium mellioti* (LiphaTech Inc., Milwaukee, WI), and for each half-sib family, approximately 10 seeds were planted in a 10 cm^2 plastic pot containing vermiculite. For each replication, the 32 half-sib families were randomly arranged in two plastic greenhouse flats, with 16 pots per flat. For experiments with race 1 isolate MF-1, each flat also contained a pot planted with the standard highly resistant check population WAPH-1 and also a pot planted with the standard susceptible check alfalfa cultivar Saranac (Fitzpatrick et al., 1998). For experiments with race 2 isolate NC-1, each flat also contained a pot planted with the standard highly resistant check population WAPH-5 and also a pot planted with WAPH-1, a standard susceptible check population for resistance to *A. euteiches* race 2 (Fitzpatrick et al., 1998).

Approximately 5 days post emergence (DPE), each pot was thinned to eight seedlings per pot. Seedlings were grown in a greenhouse with 16 hr daylength at

Table 1. Means[a] and ranges of disease severity index ratings[b] observed in 2002 and 2003 for 32 half-sib families each of alfalfa cultivars Depend +EV, 3452 ML, and Affinity + Z inoculated with *Aphanomyces euteiches* race 1 isolate MF-1 and *A. euteiches* race 2 isolate NC-1. The Spearman rank correlations (ρ) between family means observed in 2002 and 2003 are presented.

	A. euteiches MF-1 (Race 1)				
	2002		2003		
Population	Mean	Range	Mean	Range	ρ (Prob > |ρ|)
Affinity + Z	2.94	1.35–3.73	3.63	2.88–4.17	0.54 (<0.0001)
Depend + EV	2.85	1.75–3.69	3.52	2.25–4.15	0.60 (<0.0001)
3452 ML	2.77	1.75–3.56	3.59	2.60–4.10	0.68 (<0.0001)
	A. euteiches NC-1 (Race 2)				
	2002		2003		
Population	Mean	Range	Mean	Range	ρ (Prob > |ρ|)
Affinity + Z	3.81	3.38–4.13	3.90	3.67–4.21	0.21 (0.0042)
Depend + EV	3.07	2.75–3.52	3.64	3.40–3.94	0.10 (0.1721)
3452 ML	3.27	2.94–3.73	3.54	3.31–3.90	0.22 (0.0019)

[a] Data is presented in each column for an analysis of 32 half-sib families for each population. N = 48 plants for each half-sib family.
[b] 1 = no necrosis of roots and hypocotyls; 2 = slight necrosis of roots and hypocotyls; 3 = necrosis of roots and lower hypocotyl, slight chlorosis of cotyledons, and moderate stunting of stem; 4 = extensive necrosis of roots, hypocotyls and cotyledons, and severe stunting of stem, and 5 = dead seedling.

20 to 24 °C. Zoospores of each isolate of *A. euteiches* were produced in a mineral salt solution as described by Llanos and Lockwood (1960). At 7 DPE the vermiculite was saturated with water and each seedling was inoculated with 1 ml of a 500 zoospore/ml suspension by pipetting the suspension to the base of the stem. The pots were then flooded for 5 days. Seven days after inoculation, all pots were drenched with a nutrient solution (Miracle-Gro, The Scotts Co., Columbus, OH).

Fourteen days after inoculation, disease severity index (DSI) ratings were done on the surviving seedlings using an integer scale from 1–5 as follows: 1 = no necrosis of roots and hypocotyls; 2 = slight necrosis of roots and hypocotyls; 3 = necrosis of roots and lower hypocotyl, slight chlorosis of cotyledons, and moderate stunting of stem; 4 = extensive necrosis of roots, hypocotyls and cotyledons, and severe stunting of stem, and 5 = dead seedling (Fitzpatrick et al., 1998).

Estimation of heritability of resistance

Heritability on a half-sib progeny means basis (h^2_{pfm}) was calculated for resistance to *A. euteiches* according to Knapp et al. (1985) using the formula $h^2_{pfm} = 1 - M_2/M_1$. For estimates based on data from a single year (2002 or 2003), M_2 = error mean square and M_1 = family mean square. For estimates based on a combined analysis of data from both years, M_2 = family × year effects mean square and M_1 = family mean square. Exact 90% confidence intervals were calculated to determine the precision of each heritability estimate on a progeny means basis. The lower 90% confidence limit was defined as $1 - [(M_1/M_2) \times F_{1-\alpha/2:df2,df1}]^{-1}$ and the upper 90% confidence limit defined as $1 - [(M_1/M_2) \times F_{\alpha/2:df2,df}]^{-1}$ (Knapp et al., 1985). All statistical analyses were conducted using JMP software, version 5.0 (SAS Institute, Cary, NC).

Results and discussion

Comparison of resistance to race 1 and race 2 isolates of A. euteiches

Means and ranges of disease severity index (DSI) ratings are presented for the three alfalfa populations inoculated with *A. euteiches* race 1 isolate MF-1 or *A. euteiches* race 2 isolate NC-1 (Table 1). Each experiment with the race 1 isolate included six replicate pots of both the highly resistant standard check population

Table 2. Heritability estimates (h^2_{PFM}) on a progeny mean basis with 90% confidence limits (C. L.) for resistance to *Aphanomyces euteiches* race 1 isolate MF-1 and *A. euteiches* race 2 isolate NC-1 for three alfalfa populations each composed of 32 half-sib families

	A. euteiches MF-1 (Race 1)						
	2002		2003		2002–2003		
Population	h^2_{PFM}	90% C.L.	h^2_{PFM}	90% C.L.	h^2_{PFM}	90% C.L.	Width[a]
Affinity +Z	0.94	0.89–0.96	0.90	0.84–0.94	0.84	0.70–0.91	25.0
Depend +EV	0.91	0.86–0.94	0.92	0.87–0.95	0.85	0.72–0.92	23.5
3452 ML	0.90	0.84–0.94	0.93	0.89–0.95	0.90	0.82–0.95	14.4
	A. euteiches NC-1 (Race 2)						
	2002		2003		2002–2003		
Population	h^2_{PFM}	90% C.L.	h^2_{PFM}	90% C.L.	h^2_{PFM}	90% C.L.	Width
Affinity +Z	0.72	0.53–0.81	0.44	0.08–0.64	0.64	0.38–0.80	65.6
Depend +EV	0.73	0.55–0.82	0.29	0–0.54	0.62	0.39–0.76	59.7
3452 ML	0.85	0.75–0.90	0.51	0.18–0.68	0.66	0.39–0.82	65.2

[a] Expressed as the ratio (%) of the confidence limit width relative to the heritability point estimate for a combined analysis of 2002–2003.

WAPH-1 and the susceptible standard check population Saranac. For experiments in 2002 with Depend +EV, 3452 ML, and Affinity +Z, the mean DSI ratings for WAPH-1 were 2.0, 1.88 and 1.85, respectively, while the mean DSI ratings for Saranac were 3.89, 3.83, and 3.98, respectively. For experiments conducted in 2003 with Depend +EV, 3452 ML, and Affinity +Z, the mean DSI ratings for WAPH-1 were 2.26, 2.15 and 1.91, respectively, while the mean DSI ratings for Saranac were 4.38, 4.67 and 4.29, respectively. In all experiments conducted in 2002 and 2003 with the race 1 isolate, the mean DSI rating for the 32 half-sib families of each population were between the mean DSI ratings of the highly resistant and susceptible standard alfalfa check populations, which is consistent with the cultivars Depend +EV, 3452 ML and Affinity +Z all being rated in standardized tests as resistant to *A. euteiches* race 1 (Alfalfa Council, 2002). Mean DSI ratings of all three populations were higher in 2003 than in 2002 (Table 1). The Spearman rank correlation between mean DSI values observed in 2002 and 2003 among the 32 half-sib families was positive and significant ($p < 0.05$) for all three populations (Table 1).

Each experiment with race 2 isolate NC-1 included six replicate pots of both the highly resistant standard check population WAPH-5 and the susceptible standard check population WAPH-1. For experiments in 2002 with Depend +EV, 3452 ML, and Affinity +Z the mean DSI ratings for WAPH-5 were 1.44, 2.31 and 2.08, respectively, while the mean DSI ratings for WAPH-1 were 3.77, 3.94, and 4.13, respectively. For experiments in 2003 with Depend +EV, 3452 ML, and Affinity +Z the mean DSI ratings for WAPH-5 were 1.88, 1.65 and 2.38, respectively, while the mean DSI ratings for WAPH-1 were 4.02, 3.87, and 4.31, respectively. The difference in mean DSI ratings for WAPH-1 between tests with race 1 isolate MF-1, for which WAPH-1 is a highly resistant check, and tests with race 2 isolate NC-1, for which WAPH-1 is a susceptible check, clearly demonstrate the differential pathogenicity of the two races of *A. euteiches*. In all experiments conducted in 2002 and 2003 with the race 2 isolate, the mean DSI rating for the 32 half-sib families of each population were between the mean DSI ratings of the highly resistant and susceptible standard alfalfa check populations (Table 1). Mean DSI ratings of all three populations were higher in 2003 than in 2002 (Table 1). The Spearman rank correlation between mean DSI values observed in 2002 and 2003 among the 32 half-sib families was positive for all three populations, but was significant ($p < 0.05$) for only Affinity + Z and 3452 ML (Table 1). In both 2002 and 2003, less than 5% of any population consisted of plants that were resistant (DSI = 2) (Alfalfa Council, 2002), suggesting that all three populations can be considered to be susceptible to *A. euteiches* race 2 isolate NC-1.

Heritability of resistance to race 1 and race 2 isolates of A. euteiches

Heritability estimates on a half-sib progeny mean basis (h^2_{PFM}) for resistance (response) to both race 1 and race 2 of *A. euteiches* are presented for data obtained in 2002 and 2003 and also for a combined analysis of data from both years (Table 2). Significant ($p < 0.05$) effects attributed to differences between half-sib families for DSI in response to infection by *A. euteiches* race 1 isolate MF-1were observed for all three populations in both 2002 and 2003. Heritability estimates for resistance to *A. euteiches* race 1 isolate MF-1 based on a single year of data were high ($h^2_{PFM} = 0.90$) for all three different populations (Table 2) for both 2002 and 2003.

Significant ($p < 0.05$) effects attributed to differences between half-sib families for DSI in response to infection by *A. euteiches* race 2 isolate NC-1were observed for all three populations in 2002. In 2003, significant family effects in response to race 2 isolate NC-1 were only observed for Affinity + Z and 3452 ML. Heritability estimates for resistance to race 2 isolate MF-1 based on a single year of data were high ($h^2_{PFM} = 0.72$) for all three different populations (Table 2) for 2002, but did not exceed 0.51 in 2003. These results suggest that environmental variance was higher in 2003 than in 2002, although considerable effort was made to keep greenhouse conditions uniform during the experiments conducted in both years. Environmental factors effecting the expression of disease in alfalfa caused by *A. euteiches* include temperature and humidity (Grau, 1990). The temperature range (20–24 °C) in the greenhouse used for this study was controlled by an automated heating and cooling system, but watering was applied manually. Consequently, it is probable that humidity was an important contributor to environmental variance encountered in this study.

Heritability estimates based on data from a single environment or year should be considered biased towards maximum estimates of heritability (Nguyen & Sleper, 1983; Nyquist, 1991). The upward bias arises from the genetic variance among half-sib families including non-additive components such as family $\times$ year, family $\times$ environment and family $\times$ year $\times$ environment interaction variances (Nguyen & Sleper, 1983). Accordingly, reliance on these estimates to calculate expected gain from selection would result in overestimated gains. Estimates of heritability obtained using data obtained over multiple years are more robust than estimates based on a single year (Nyquist, 1991).

A combined analysis of data generated over both years indicated that significant effects ($p < 0.05$) attributed to family x year interactions were observed for all three populations in response to infection by both race 1 and race 2 isolates of *A. euteiches*. Heritability estimates of resistance to race 1 were slightly lower when estimates were made based on a combined analysis of both years (Table 2). However, these estimates were high and quite similar for all three populations, ranging from 0.84–0.90. Heritability estimates of resistance to race 2 based on a combined analysis of data over both years, although lower than estimates observed for race 1, were moderately high and quite similar for all three populations, ranging from 0.62–0.66.

The estimates of heritability reported in this study were based on populations derived from the cultivars Depend +EV, Affinity + Z and 3542 ML, all of which have been previously selected for resistance to race 1 of *A. euteiches*. These results suggest that considerable additive genetic variance is still available in these populations for making further improvement in resistance to *A. euteiches* race 1. The heritability estimates for resistance to race 2 will be particularly useful for alfalfa improvement programs, since resistance to race 2 is currently lacking in the great majority of alfalfa cultivars (Alfalfa Council, 2002). These estimates suggest that levels of resistance to *A. euteiches* race 2 can be enhanced by selection among elite alfalfa materials that have previously been selected for resistance to *A. euteiches* race 1.

Similarities observed among the different populations for estimates of heritability may reflect parental sources that are shared among the alfalfa cultivars used to produce the three populations. The three cultivars have similar percentages of contributions from different *Medicago* germplasm sources (Barnes et al., 1977). Among the three cultivars, the contribution from Flemish, *M. varia* and Turkistan germplasm ranged from 33–38%, 17–21% and 13–17% respectively. The cultivars were all selected for resistance to multiple diseases, including several wilt diseases and root rot caused by *A. euteiches* race 1.

Our estimates of heritability based on two years of data (Table 2) were considerably higher than the estimate of 0.30 for heritability of resistance in pea to *A. euteiches* (Pilet-Nayel et al., 2003). This is despite the fact that the populations we examined in this study were derived from three cultivars that had been previously selected for resistance to *A. euteiches* race 1. A lower estimate of heritability in peas may reflect differences in the relative contribution of environmental variance towards total phenotypic variance encountered in our study and that conducted by Pilet-Nayal et al. (2003). The heritability estimate for pea was based on two years of experiments conducted in a field disease screening nursery (Pilet-Nayel et al., 2003). It is probable that more environmental variance was encountered in the pea study than was encountered in our experiments conducted under greenhouse conditions. A greater contribution of environmental variance towards total phenotypic variance would result in lower estimates of heritability.

The width of the heritability estimate (Table 2), expressed as the ratio (%) of the confidence interval width relative to the heritability point estimate, can be considered an indicator of the precision of the estimate (Ray et al., 1999). The widths of heritability estimates for resistance to *A. euteiches* in pea ranged from 62–80% (Pilet-Nayel et al., 2003). Heritability estimates calculated in this study for resistance to *A. euteiches* race 1 were more precise, with the width of the estimates ranging from 14.4–25.0% (Table 2). The width of the estimates for resistance in alfalfa to *A. euteiches* race 2 (Table 2) were similar to those observed previously for pea. The use of exact confidence intervals is preferred over the use of approximate standard errors for estimating precision of heritability estimates, since heritability estimates are not symmetrically distributed, an assumption implicit in the use of standard errors (Knapp et al., 1985). The heritability estimates reported in this study will be particularly useful in predicting gain from selection for resistance to *A. euteiches* race 2 among elite alfalfa materials that have previously been selected for resistance to race 1 of *A. euteiches*.

References

Alfalfa Council, 2002. Fall Dormancy and Pest Resistance Ratings for Alfalfa Varieties. Alfalfa Council, Kansas City, MO.

Barnes, D.K., E.T. Bingham, R.P. Murphy, O.J. Hunt, D.F. Beard, W.H. Skrdla & L.R. Teuber, 1977. Alfalfa germplasm in the United States: Genetic vulnerability, use, improvement and maintenance. U.S. Dept Agric Res Tech Bull No. 1571.

Delwiche, P.A., C.R. Grau, E.B. Holub & J.B. Perry, 1987. Characterization of *Aphanomyces euteiches* isolates recovered from alfalfa in Wisconsin. Plant Dis 71: 155–161.

Fitzpatrick, S., J. Brummer, B. Hudelson, D. Malvick & C.R. Grau, 1998. Standard Test: Aphanomyces Root Rot Resistance (Races 1 and 2). North American Alfalfa Improvement Conference. On-line publication. http: //www.naaic.org.

Grau, C.R., 1990. Aphanomyces root rot. In: D.L. Stuteville & D.C. Erwin (Eds.), Compendium of Alfalfa Diseases, pp. 10–11. American Phytopathological Society Press, St. Paul, MN.

Grau, C.R., A.M. Muehlchen & J.E. Tofte, 1991. Variability in virulence of *Aphanomyces euteiches*. Plant Dis 75: 1153–1156.

Holub, E.B., C.R. Grau & J.L. Parke, 1991. Evaluation of the *forma specialis* concept in *Aphanomyces euteiches*. Mycol Res 95: 147–157.

Knapp, S.J., W.W. Stroup & W.M. Ross, 1985. Exact confidence intervals for heritability on a progeny mean basis. Crop Sci 25: 192–194.

Llanos, C. & J.L. Lockwood, 1960. Factors affecting zoospore production by *Aphanomyces euteiches*. Phytopathology 50: 826–830.

Munkvold, G.P., W.M. Carlton, E.C. Brummer, J.R. Meyer, D.J. Undersander & C.R. Grau, 2001. Virulence of *Aphanomyces euteiches* isolates from Iowa and Wisconsin and benefits of resistance to *A. euteiches* in alfalfa cultivars. Plant Dis 85: 328–333.

Nguyen, H.T. & D.A. Sleper, 1983. Theory and application of half-sib matings in forage grass breeding. Theor Appl Genet 64: 187–196.

Nyquist, W.E., 1991. Estimation of heritability and predictions of selection response in plant populations. Crit Rev Plant Sci 10: 235–322.

Papavizas, G.C. & W.A. Ayers, 1974. Aphanomyces species and their root diseases in pea and sugarbeet. US Dept Agric Res Tech Bull No. 1485.

Pfender, W.F. & D.J. Hagedorn, 1982. *Aphanomyces euteiches* f. sp. *phaseoli*, a causal agent of bean root and hypocotyl rot. Phytopathology 72: 306–310.

Pilet-Nayel, M.L., F.J. Muehlbauer, R.J. McGee, J.M. Kraft, A. Baranger & C.J. Coyne, 2002. Quantitative trait loci for partial resistance to Aphanomyces root rot in pea. Theor Appl Genet 106: 28–39.

Ray, I.M., M.S. Townsend, C.M. Muncy & J.A. Henning, 1999. Heritabilities of water use efficiency traits and correlations with agronomic traits in water stressed alfalfa. Crop Sci 39: 494–498.

Shehata, M.A., D.W. Davis & F.L. Pfleger, 1983. Breeding for resistance to *Aphanomyces euteiches* root rot and *Rhizoctonia solani* stem rot in peas. J Am Soc Hort Sci 108: 1080–1085.

Vincelli, P., J. Henning, T. Hendrick, J. Brown, L.J. Osborne, B. Prewitt, V. Shields, D. Sorrell, K.D. Strohmeier, R. Tackett & J.W. Wyles, 2000. Improved seedling health, yield, and stand persistence with alfalfa resistant to Aphanomyces root rot. Agron J 92: 1071–1076.

Wiersma, D.W., C.R. Grau & D.J. Undersander, 1995. Alfalfa cultivar performance with differing levels of resistance to Phytophthora and Aphanomyces root rots. J Prod Agric 8: 259–264.

Wiersma, D.W., D.J. Undersander & C.R. Grau, 1997. Root heave of alfalfa cultivars with differing levels of resistance to Aphanomyces root rot. Agron J 89: 148–150.